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Apoptosis coordinates with proliferation and differentiation during human hair follicle morphogenesis

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Summary

Background : Apoptosis sculptures the most complicated skin appendage, feathers, out of epidermal layers by playing a variety of roles (1). Human hair follicle formation is different from feathers in growth direction and pattern formation of proliferative zone.
Objective : To delineate the apoptotic events together with proliferation and differentiation in developing human hair follicle and emphasis on the mechanism and biological meaning of epidermal hair canal.
Methods : We used TUNEL to examine apoptosis and Ki-67, involucrin, filaggrin immuno-localization to examine proliferation and differentiation.
Results : In hair germs, apoptosis was diffuse in periderm, basal keratinocytes, and mesenchymal cells with high Ki-67 expression, but spared follicular germinative cells with low Ki-67 and high bcl-2 expression. In hair pegs, apoptosis was active in high Ki-67 expression area, like outer root sheath, hair follicle sheath, but spared dermal papilla with low Ki-67 and high bcl-2 expression. In bulbous pegs, apoptosis appeared in companion layer, precortical area, inner root sheath and outer root sheath, but spared bulge area with high bcl-2 expression. Apoptosis resulted in epidermal and subepidermal hair canal formation. Filaggrin and involucrin were expressed in the lining cells of hair canal. $CD1a^+$ cells were densely distributed alone the hair canal before its opening
Conclusion : During human hair follicle morphogenesis, apoptosis coordinates with proliferation to shape the growth zone, creates space to free the hair shaft from follicular wall, and directs a driving force on hair shaft extension. Apoptosis accom-

Abbreviations: TUNEL, TdT-mediated dUTP nick end label; EGA, estimated gestational age

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panies the terminal differentiation of epidermal hair canal. The bottom becomes interfollicular epidermis after roof shedding and hair exposure. Langerhans cells also populate in the hair canal before its opening. The biological meaning of epidermal hair canal is supposed to prepare the barrier when hair perturbing the intact of epidermis.

1. Introduction

Hair, feathers, teeth, beaks, nails, and several glands (e.g., mammary, sweat, salivary, and lacrimal glands) are all derivatives of the ectoderm. Morphogenesis of these ectodermal organs is orchestrated by common developmental processes including induction, cell fate specification, proliferation, differentiation, apoptosis, epithelial cycling and intimate interactions with the mesenchyme [2]. Using murine or chicken models, the role of cell proliferation [3] and differentiation [4,5] have been studied. Recently we used feathers as a model to show that apoptosis play important roles in sculpturing skin appendages out of the epidermal layers [1].

Human hair follicles are derived from ectoderm (epidermis) and mesoderm (dermis). At the end of the third month of estimated gestational age (EGA), crowding of basal cells with elongated nuclei (placode or pregerm) appeared as the follicular germinative cells. Unlike feather, the growth zone in feather bud generates an upward growth first and a downward invagination later, growth zone in human hair germ induces a downward growth first to form the hair peg. At the end of the fourth month EGA, two or three swellings appeared at the posterior side of epidermal column. The lower most forms the bulge, which acts as the attachment point of arrector pili muscle and also contains hair follicle stem cells. The middle swelling develops into the sebaceous gland. The uppermost swelling, if present, either involutes or develops into an apocrine gland. As the bulbous peg stage is reached, differentiation occurs in the lower and upper portions of the hair follicle and in the overlying epidermis. Both hair shaft and inner root sheath form concomitantly through upward terminal differentiation of transient amplifying matrix cells [6,7]. There is a sliding plane between the opposite migration layers of inner and outer root sheath, the companion layer [8]. When reaching the isthmus level (above bulge and below entrance of sebaceous duct), the inner root sheath and companion layer will shed in the hair canal. Hair shaft does not penetrate onto the skin surface directly, but is buried horizontally in the epidermal compartment for several weeks until releasing to the outside world [9]. How apoptosis works in the morphogenesis of human hair follicle and what their functional meanings are have not been addressed in detail.

In this study, we examined the apoptotic events by TdT-mediated dUTP nick end label (TUNEL) staining [10]. This technique enables the identification of apoptotic cells in restricted regions [11]. We also studied the relation between apoptosis and proliferation and relation between apoptosis and terminal differentiation. Our data revealed apoptosis was efficiently applied temporally and spatially in human hair follicle morphogenesis. The biologic meaning that hair shaft buried in epidermal hair canal is to wait the terminal differentiation of interfollicular epidermis. Apoptosis is not only the main mechanism to form the hair canal, but also related to the barrier function formation.

2. Materials and methods

2.1. Human specimens

Skin was sampled from human embryos and fetuses, ages 7—34 weeks EGA. Human specimen age was determined from maternal data and measurements of foot and crown-rump lengths when possible, or comparative histologic appearance of the epidermis. Specimens were sampled from the scalp. All of the specimens were formalin-fixed, and paraffin-embedded.

2.2. TUNEL staining

TUNEL staining was performed following the recommendations of the manufacturer (In Situ Cell Death Detection, POD Kit (Roche)). Deparaffinized tissue sections were treated with proteinase K 20 ug/ml, 15 min at room temperature to strip proteins from nuclei and rinses three times (3 min each) with deionized water (DDW). Inactivate endogeneous peroxidase by incubation with 0.3% H_2O_2. Methyl green (DAKO) was used for counterstaining when necessary.

2.3. Immunohistochemistry

Immunohistochemistry was performed as described [12] with the following modification. After dewaxing